

I/WE CLAIM:

1. A method for detecting a target nucleic acid in a sample comprising:

- (a) contacting the target nucleic acid with a circular oligonucleotide probe under conditions that allow hybridization between complementary sequences in the target nucleic acid and the circular oligonucleotide probe;
- (b) adding at least one forward primer comprising a sequence complementary to a portion of the circular oligonucleotide probe;
- (c) adding an oligonucleotide primer pair comprising a first primer and a second primer, wherein
  - (i) the first primer of the pair comprises (A) a first sequence that is substantially identical to a portion of the circular oligonucleotide probe, (B) a second sequence that is complementary to the second primer of the pair, and (C) a signal generating moiety;
  - (ii) the second primer of the pair comprises (A) a sequence that is complementary to the first primer and (B) a moiety capable of quenching, masking or inhibiting the activity of the signal generating moiety when located adjacent to, or in close proximity to the signal generating moiety; and
  - (iii) when the first primer and the second primer are bound to one another, the signal is inhibited;
- (d) adding at least one reverse primer comprising a sequence that is substantially identical to a portion of the circular oligonucleotide probe;

(e) adding a DNA polymerase; and

(f) amplifying the circular oligonucleotide probe thereby separating the signal generating moiety and the quenching, masking or inhibitory moiety to generate a signal, wherein detection thereof indicates the presence of the target nucleic acid in the sample.

2. The method of claim 1, whereby the circular oligonucleotide probe is formed by ligating the 3' and 5' ends of a linear oligonucleotide probe, comprising 3' and 5' regions complementary to adjacent sequences in the target nucleic acid under conditions that allow hybridization between complementary sequences in the target nucleic acid and the linear oligonucleotide probe.

3. The method of claim 1, wherein the sequence of the reverse primer is SEQ ID NO: 49.

4. The method of claim 1, wherein the sequence of the first primer of the oligonucleotide primer pair is SEQ ID NO: 43 and the sequence of the second primer of the oligonucleotide primer pair is SEQ ID NO: 44.

5. The method of claim 1, wherein the signal generating moiety is a fluorescent agent.

6. The method of claim 1, wherein the signal generating moiety is a chemiluminescent agent.

7. The method of claim 1, wherein the signal generating moiety is an enzyme or enzyme substrate.

8. The method of claim 1, wherein the circular probe is amplified using an amplification method selected from the group consisting of polymerase chain reaction, strand displacement amplification, transcription mediated amplification, RAM and primer extension.

9. The method of claim 8, wherein the amplification method is RAM.

10. A kit for detecting a target nucleic acid, the kit comprising:

(a) an oligonucleotide probe comprising regions that are complementary in sequence to the target nucleic acid;

- (b) at least one forward oligonucleotide primer comprising a sequence that is complementary to a portion of the oligonucleotide probe;
- (c) an oligonucleotide primer pair comprising a first primer and a second primer, wherein
  - (i) the first primer comprises (A) a first sequence that is substantially identical to a portion of the oligonucleotide probe, (B) a second sequence that is complementary to the second primer of the pair, and (C) a signal generating moiety;
  - (ii) the second primer of the pair comprises (A) a sequence that is complementary to the first primer and (B) a moiety capable of quenching, masking or inhibiting the activity of the signal generating moiety when located adjacent to, or in close proximity to the signal generating moiety; and
  - (iii) when the first primer and the second primer are bound to one another, the signal is inhibited; and
- (d) at least one reverse oligonucleotide primer comprising a sequence that is substantially identical to a portion of the oligonucleotide probe.

- 11. The kit of claim 10, wherein the kit further contains instructions for detecting a target nucleic acid.
- 12. The kit of claim 10, wherein the kit further comprises a DNA polymerase.
- 13. A method for detecting a target nucleic acid in a sample comprising:

- (a) contacting the target nucleic acid with a circular oligonucleotide probe under conditions that allow hybridization between complementary sequences in the target nucleic acid and the circular oligonucleotide probe;
- (b) adding at least one first oligonucleotide primer pair comprising a first primer and a second primer, wherein
  - (i) the first primer of the first primer pair comprises (A) a first sequence that is complementary to a portion of the circular oligonucleotide probe, (B) a second sequence that is complementary to the second primer of the first primer pair, and (C) a signal generating moiety;
  - (ii) the second primer of the pair comprises (A) a sequence that is complementary to the first primer of the first primer pair and (B) a moiety capable of quenching, masking or inhibiting the activity of the signal generating moiety when located adjacent to, or in close proximity to the signal generating moiety; and
  - (iii) when the first primer and the second primer of the first primer pair are bound to one another, the signal is inhibited;
- (c) adding at least one second oligonucleotide primer pair comprising a first primer and a second primer of the second primer pair, wherein
  - (i) the first primer of the second primer pair comprises (A) a first sequence that is substantially identical to a portion of the circular oligonucleotide probe, (B) a second sequence that is complementary to the second primer of the second primer pair, and (C) a signal generating moiety; and

(ii) the second primer of the second primer pair comprises (A) a sequence that is complementary to the first primer of the second primer pair and (B) a moiety capable of quenching, masking or inhibiting the activity of the signal generating moiety when located adjacent to, or in close proximity to the signal generating moiety;

(d) adding a DNA polymerase; and

(e) amplifying the circular oligonucleotide probe thereby separating the signal generating moiety and the quenching, masking or inhibitory moiety to generate a signal, wherein detection thereof indicates the presence of the target nucleic acid in the sample.

14. The method of claim 13, whereby the circular oligonucleotide probe is formed by ligating the 3' and 5' ends of a linear oligonucleotide probe, comprising 3' and 5' regions complementary to adjacent sequences in the target nucleic acid under conditions that allow hybridization between complementary sequences in the target nucleic acid and the linear oligonucleotide probe.

15. The method of claim 13, wherein the signal generating moiety is a fluorescent agent.

16. The method of claim 13, wherein the signal generating moiety is a chemiluminescent agent.

17. The method of claim 13, wherein the signal generating moiety is an enzyme or enzyme substrate.

18. The method of claim 13, wherein the circular probe is amplified using an amplification method selected from the group consisting of polymerase chain reaction, strand displacement amplification, transcription mediated amplification, RAM and primer extension.

19. The method of claim 18, wherein the amplification method is RAM.

20. A kit for detecting a target nucleic acid, the kit comprising:

(a) an oligonucleotide probe comprising regions that are complementary in sequence to the target nucleic acid;

(b) at least one first oligonucleotide primer pair comprising a first primer and a second primer, wherein

(i) the first primer of the first primer pair comprises (A) a first sequence that is complementary to a portion of the circular oligonucleotide probe, (B) a second sequence that is complementary to the second primer of the first primer pair, and (C) a signal generating moiety;

(ii) the second primer of the first primer pair comprises (A) a sequence that is complementary to the first primer of the first primer pair and (B) a moiety capable of quenching, masking or inhibiting the activity of the signal generating moiety when located adjacent to, or in close proximity to the signal generating moiety; and

(iii) when the first primer and the second primer of the first primer pair are bound to one another, the signal is inhibited; and

(c) at least one second oligonucleotide primer pair comprising a first primer and a second primer, wherein

(i) the first primer of the second primer pair comprises (A) a first sequence that is substantially identical to a portion of the circular oligonucleotide probe, (B) a second sequence that is complementary to the second primer of the second primer pair, and (C) a signal generating moiety; and

(ii) the second primer of the second primer pair comprises (A) a sequence that is complementary to the first primer of the second primer pair and (B) a moiety capable of quenching, masking or inhibiting the activity of the signal generating moiety when located adjacent to, or in close proximity to the signal generating moiety.

21. The kit of claim 20, wherein the kit further contains instructions for detecting a target nucleic acid.

22. The kit of claim 20, wherein the kit further comprises a DNA polymerase.

23. A method for detecting a target nucleic acid in a sample comprising:

(a) contacting the target nucleic acid with a circular oligonucleotide probe under conditions that allow hybridization between complementary sequences in the target nucleic acid and the circular oligonucleotide probe;

(b) adding at least one multiple oligonucleotide primer comprising a first primer, a second primer and a third primer, wherein

(i) the first primer of the multiple oligonucleotide primer comprises (A) a first sequence that is complementary to a portion of the circular oligonucleotide probe, (B) a second sequence that is complementary to the second primer of the multiple oligonucleotide primer, and (C) a third sequence that is complementary to the third primer of the multiple oligonucleotide primer;

(ii) the second primer of the multiple oligonucleotide primer comprises (A) a sequence that is complementary to the first primer of the multiple oligonucleotide primer and (B) a signal generating moiety;

(iii) the third primer of the multiple oligonucleotide primer comprises (A) a sequence that is complementary to the first primer of the multiple oligonucleotide primer and (b) a moiety capable of quenching, masking or inhibiting the activity of the signal generating moiety when located adjacent to, or in close proximity to the signal generating moiety; and

(iv) when the first, second and third primers of the multiple oligonucleotide primer are bound to one another, the signal is inhibited;

(c) adding at least one reverse primer comprising a sequence that is substantially identical to a portion of the circular probe;

(d) adding a DNA polymerase; and

(e) amplifying the circular oligonucleotide probe thereby separating the signal generating moiety and the quenching, masking or inhibitory moiety to generate a signal, wherein detection thereof indicates the presence of the target nucleic acid in the sample.

24. The method of claim 23, whereby the circular oligonucleotide probe is formed by ligating the 3' and 5' ends of a linear oligonucleotide probe, comprising 3' and 5' regions complementary to adjacent sequences in the target nucleic acid under conditions that allow hybridization between complementary sequences in the target nucleic acid and the linear oligonucleotide probe.

25. The method of claim 23, wherein the signal generating moiety is a fluorescent agent.

26. The method of claim 23, wherein the signal generating moiety is a chemiluminescent agent.

27. The method of claim 23, wherein the signal generating moiety is an enzyme or enzyme substrate.



28. The method of claim 23, wherein the circular probe is amplified using an amplification method selected from the group consisting of polymerase chain reaction, strand displacement amplification, transcription mediated amplification, RAM and primer extension.

29. The method of claim 28, wherein the amplification method is RAM.

30. A kit for detecting a target nucleic acid, the kit comprising:

- (a) an oligonucleotide probe comprising regions that are complementary in sequence to the target nucleic acid;

- (b) at least one multiple oligonucleotide primer comprising a first primer, a second primer and a third primer; wherein

- (i) the first primer of the multiple oligonucleotide primer comprises (A) a first sequence that is complementary to a portion of the circular oligonucleotide probe, (B) a second sequence that is complementary to the second primer of the multiple oligonucleotide primer, and (C) a third sequence that is complementary to the third primer of the multiple oligonucleotide primer;

- (ii) the second primer of the multiple oligonucleotide primer comprises (A) a sequence that is complementary to the first primer of the multiple oligonucleotide primer and (B) a signal generating moiety;

- (iii) the third primer of the multiple oligonucleotide primer comprises (A) a sequence that is complementary to the first primer of the multiple oligonucleotide primer and (b) a moiety capable of quenching, masking or inhibiting the activity of the signal generating moiety when located adjacent to, or in close proximity to the signal generating moiety; and

(iv) when the first, second and third primers of the multiple oligonucleotide primer are bound to one another, the signal is inhibited; and

(c) at least one reverse primer, comprising a sequence that is substantially identical to a portion of the circular probe.

31. The kit of claim 30, wherein the kit further contains instructions for detecting a target nucleic acid.

32. The kit of claim 30, wherein the kit further comprises a DNA polymerase.

33. A method for detecting a target nucleic acid in a sample comprising:

(a) contacting the target nucleic acid with a circular oligonucleotide probe under conditions that allow hybridization between complementary sequences in the target nucleic acid and the circular oligonucleotide probe;

(b) adding at least one multiple oligonucleotide primer comprising a first primer, a second primer and a third primer, wherein

(i) the first primer of the multiple oligonucleotide primer comprises (A) a first sequence that is substantially identical to a portion of the circular oligonucleotide probe, (B) a second sequence that is complementary to the second primer of the pair, and (C) a third sequence that is complementary to the third primer of the multiple oligonucleotide primer;

(ii) the second primer of the multiple oligonucleotide primer comprises (A) a sequence that is complementary to the first primer of the multiple oligonucleotide primer and (B) a signal generating moiety;

(iii) the third primer of the multiple oligonucleotide primer comprises (A) a sequence that is complementary to the first primer of the multiple oligonucleotide primer and (b) a moiety capable of quenching, masking or inhibiting the activity of the signal generating moiety when located adjacent to, or in close proximity to the signal generating moiety; and

(iv) when the first, second and third primers of the multiple oligonucleotide primer are bound to one another, the signal is inhibited; and

(c) adding at least one forward primer comprising a sequence that is complementary to a portion of the circular probe;

(d) adding a DNA polymerase; and

(e) amplifying the circular oligonucleotide probe thereby separating the signal generating moiety and the quenching, masking or inhibitory moiety to generate a signal, wherein detection thereof indicates the presence of the target nucleic acid in the sample.

34. The method of claim 33, whereby the circular oligonucleotide probe is formed by ligating the 3' and 5' ends of a linear oligonucleotide probe, comprising 3' and 5' regions complementary to adjacent sequences in the target nucleic acid under conditions that allow hybridization between complementary sequences in the target nucleic acid and the linear oligonucleotide probe.

35. The method of claim 33, wherein the signal generating moiety is a fluorescent agent.

36. The method of claim 33, wherein the signal generating moiety is a chemiluminescent agent.

37. The method of claim 33, wherein the signal generating moiety is an enzyme or enzyme substrate.

38. The method of claim 33, wherein the circular probe is amplified using an amplification method selected from the group consisting of polymerase chain reaction, strand displacement amplification, transcription mediated amplification, RAM and primer extension.

39. The method of claim 38, wherein the amplification method is RAM.

40. A kit for detecting a target nucleic acid, the kit comprising:

(a) an oligonucleotide probe comprising regions that are complementary in sequence to the target nucleic acid;

(b) at least one multiple oligonucleotide primer comprising a first primer, a second primer and a third primer, wherein

(i) the first primer of the multiple oligonucleotide primer comprises (A) a first sequence that is substantially identical to a portion of the circular oligonucleotide probe, (B) a second sequence that is complementary to the second primer of the multiple oligonucleotide primer, and (C) a third sequence that is complementary to the third primer of the multiple oligonucleotide primer;

(ii) the second primer of the multiple oligonucleotide primer comprises (A) a sequence that is complementary to the first primer of the multiple oligonucleotide primer and (B) a signal generating moiety;

(iii) the third primer of the multiple oligonucleotide primer comprises (A) a sequence that is complementary to the first primer of the multiple oligonucleotide primer and (b) a moiety capable of quenching, masking or inhibiting the activity of the signal generating moiety when located adjacent to, or in close proximity to the signal generating moiety; and

(iv) when the first, second and third primers of the multiple oligonucleotide primer are bound to one another, the signal is inhibited; and

(c) at least one forward primer comprising a sequence that is complementary to a portion of the circular probe.

41. The kit of claim 40, wherein the kit further contains instructions for detecting a target nucleic acid.

42. The kit of claim 40, wherein the kit further comprises a DNA polymerase.

43. A method for amplifying a circular nucleic acid sequence, said method comprising:

(a) contacting the circular nucleic acid sequence with at least one forward primer comprising a sequence complementary to a portion of the circular nucleic acid sequence;

(b) adding an oligonucleotide primer pair comprising a first primer and a second primer, wherein

(i) the first primer of the pair comprises (A) a first sequence that is substantially identical to a portion of the circular nucleic acid sequence, and (B) a second sequence that is complementary to the second primer of the pair;

(ii) the second primer of the pair comprises a sequence that is complementary to the first primer of the pair;

(c) adding at least one reverse primer comprising a sequence that is substantially identical to a portion of the circular oligonucleotide probe;

(d) adding a DNA polymerase; and

(e) amplifying the circular nucleic acid sequence.

44. The method of claim 43, wherein the first primer of the primer pair further comprises a signal generating moiety, the second primer of the primer pair further comprises a moiety capable of quenching, masking or inhibiting the activity of the signal generating moiety when located adjacent to, or in close proximity to the signal generating moiety, and when the first primer and the second primer of the primer pair are bound to one another, the signal is inhibited.

45. The method of claim 44, said method further comprising:

contacting the circular nucleic acid sequence to a target nucleic acid under conditions that allow hybridization between complementary sequences in the target nucleic acid, wherein the amplification of the circular nucleic acid sequence separates the signal generating moiety of the first primer and the quenching, masking or inhibitory moiety of the second primer to generate a signal, wherein detection thereof indicates the presence of the target nucleic acid.

46. A method for detecting a target nucleic acid in a sample comprising:

(a) contacting the target nucleic acid with at least one oligonucleotide primer pair comprising a first primer and a second primer, wherein

(i) the first primer of the pair comprises (A) a first sequence that is complementary to a portion of the target nucleic acid, (B) a second sequence that is complementary to the second primer of the pair, and (C) a signal generating moiety;

(ii) the second primer of the pair comprises (A) a sequence that is complementary to the first primer and (B) a moiety capable of quenching, masking or inhibiting

the activity of the signal generating moiety when located adjacent to, or in close proximity to the signal generating moiety; and

(iii) when the first primer and the second primer are bound to one another, the signal is inhibited;

(b) adding at least one reverse primer comprising a sequence that is substantially identical to a portion of the target nucleic acid;

(c) adding a DNA polymerase; and

(d) amplifying the target nucleic acid thereby separating the signal generating moiety and the quenching, masking or inhibitory moiety to generate a signal, wherein detection thereof indicates the presence of the target nucleic acid in the sample.

47. The method of claim 46, wherein the signal generating moiety is a fluorescent agent.

48. The method of claim 46, wherein the signal generating moiety is a chemiluminescent agent.

49. The method of claim 46, wherein the signal generating moiety is an enzyme or enzyme substrate.

50. The method of claim 46, wherein the target nucleic acid is amplified using an amplification method selected from the group consisting of polymerase chain reaction, strand displacement amplification, transcription mediated amplification, RAM and primer extension.

51. The method of claim 50, wherein the amplification method is RAM.

52. The method of claim 50, wherein the amplification method is polymerase chain reaction.

53. A kit for detecting a target nucleic acid, the kit comprising:

(a) at least one oligonucleotide primer pair comprising a first primer and a second primer, wherein

(i) the first primer comprises (A) a first sequence that is complementary to a portion of the target nucleic acid, (B) a second sequence that is complementary to the second primer of the pair, and (C) a signal generating moiety;

(ii) the second primer of the pair comprises (A) a sequence that is complementary to the first primer and (B) a moiety capable of quenching, masking or inhibiting the activity of the signal generating moiety when located adjacent to, or in close proximity to the signal generating moiety; and

(iii) when the first primer and the second primer are bound to one another, the signal is inhibited; and

(b) at least one reverse oligonucleotide primer comprising a sequence that is substantially identical to a portion of the target nucleic acid.

54. The kit of claim 53, wherein the kit further contains instructions for detecting a target nucleic acid.

55. The kit of claim 53, wherein the kit further comprises a DNA polymerase.